Influence of Load on Order–Disorder Transitions in Collagen Fibers†

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ABSTRACT: The influence of external stress on the hydrothermal and chemical contraction of native collagen was investigated. The coincidence of isometric and isotonic data was observed. This permits the consideration of the isometric curve from the beginning of contraction to the critical point as a phase diagram of the system in coordinates of stress temperature. The existence of a certain critical tension and a corresponding critical temperature or of a critical tension and critical KCNS concentration is suggested. This result is in accordance with the predictions of the theory of order-disorder transitions in these unidimensional systems. Using the formulas of this theory we calculated the enthalpy of the hydrothermal contraction of collagen, the enrichment of salt for its chemical contraction and the cooperativity parameters for both processes.

The influence of load on the order–disorder transitions in oriented crystalline polymers and in fibrous proteins in particular was first considered theoretically by S. Ya. Frenkel[1] and by Gee[2] and Flory.[3] The point of conformational transition was found to be determined by external force. The equation connecting the heat and entropy of transition and the changes in length and external force was derived. This equation is similar to the Clausius-Clapeyron equation for the usual systems.

Birstein, Vorob'ev, and Ptitsin[4,5] drew the same conclusion as Flory and Gee from the statistical-mechanical treatment of this problem in terms of helix-random-coil transition. According to these theories one can represent this order–disorder transition schematically for the case of an idealized α helix. The following peculiarities can be noted (Figure 1). Upon the breakage of the stabilizing cooperative system of hydrogen bonds caused by temperature or changes in chemical environment the helical "spring" of the polymer tends to contract to a relatively symmetrical coil (the O–I transition). The external force must increase the transition temperature and diminish the contraction value as the observed effect is the contraction of the sample. This must hold until the linear dimensions of the deformed coil become equal to the length of the helix. Some critical load (or a critical range of loads) must fit this case; under this critical load contraction does not occur. After the next increase of load above the critical one the linear dimensions of the random coil will exceed the length of the helix and an elongation of the fiber must occur instead of shortening as well as the reducing of the transition temperature with the load. This must be the case up to the full extension of the polymer chain; in Figure 1 the corresponding β structure stabilized by cross-linking hydrogen bonds is represented.

The main aim of the present study was to develop an equilibrium method for the investigation of the contraction of fibrous proteins, and of the hydrothermal and chemical contraction of collagen in particular, and to check experimentally the real existence of the critical point for such systems.

Native collagen has been chosen by us as a subject of investigation. It has a highly ordered structure and gives an X-ray pattern with a clear-cut reflection. Rat tail tendons were tanned by formaldehyde to introduce cross-bridges between polypeptide chains to ensure the reversibility of contraction. The hydrothermal contraction, *i.e.*, the heat contraction in water medium and the contraction induced by concentrated (>1 mol/l.) water solutions of KCNS, were studied.

The measurements were made by two modes: isometric and isotonic. When the isometric mode was used the length of the sample was fixed and the dependence of the internal stress on the acting factor, which was temperature or the medium composition, was recorded. When the isotonic method was used, the external load was fixed and the dependence of the length of the sample on the acting factor was recorded correspondingly. The structural changes occuring in the contraction processes were observed by X-ray diffraction.

Both the hydrothermal contraction of collagen and its contraction induced by KCNS solutions have the nature of an order–disorder transition which is analogous to the melting of low molecular weight substances. It was evidenced by the existence of heat absorption[6] and by a small but reproducible volume increase[7] accompanying the contraction and was verified by X-ray diffraction during hydrothermal contraction of collagen. For the KCNS contraction the same result was also shown by Zaydes from X-ray studies.[8]

Figure 2 represents a series of isotonic curves of hydrothermal contraction of collagen for different external loads. The transition temperature is on the abscissa and the percentage relative contraction is on the ordinate. The values of loads are designated by the corresponding curves. It is seen from the figure that the transition temperature rises and the magnitude of contraction diminishes when the stress is increased, and according to the theoretical predictions there must be some critical load under which no contraction occurs, as is shown by the dotted line. It was impossible to determine this load directly from the experimental data as the corresponding transition temperature is higher than the boiling point of water. It can be determined, however, by extrapolation of the curve for stress *vs.* maximal relative contraction to the contraction (Figure 3); the stress in kg/cm^2 is on the abscissa, and the percentage relative contraction is on the ordinate. The estimate is 165 ± 10 kg/cm^2. Figure 3c (in which the stress is on the abscissa and the transition temperature is on the ordinate) represents the experimental isometric curves of the collagen hydrothermal contraction.

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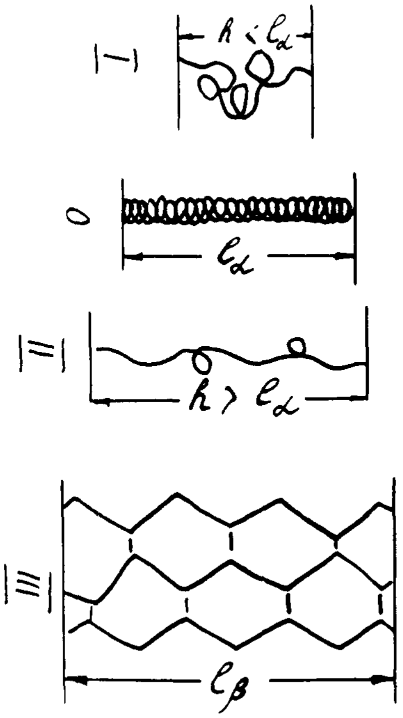

Figure 1. Possible conformational transitions for an idealized α helix under different loads. For details, see text.

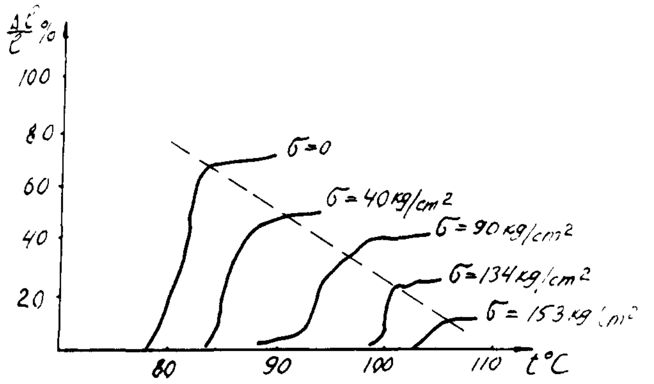

Figure 2. Hydrothermal contraction of collagen fibers at different fixed loads.

Figure 3b (the same axes) shows the transition temperatures for different isotonic curves; it is seen that the points are scattered closely along the continuous isometric curve. Thus, there is a full coincidence of isometric and isotonic data. The other coordinate of the critical point—the critical temperature—can be determined from this data by extrapolation of the straight line thus obtained to the critical stress value ($\sigma = \sigma$). Thus, the real existence of a critical point is proved for this system and its coordinates are determined.

The physical meaning of the isometric heating curve is the following. The fibers were subjected to an initial minute stretching load of 1-5 kg/cm^2. Having attained a certain temperature which corresponds to the transition under similar conditions the fiber tends to contract; due to the fact that it is fixed a longitudinal internal stress appears within it. But, as already mentioned, this increase in stress also increases the transition temperature. When this new "transition" temperature is reached the corresponding growth of internal stress again causes further increasing of the transition temperature and so on. This process will continue until some critical stress is reached which corresponds to the theoretically predicted critical external stress. Thus, the isometric heating curve represents the dependence of the temperature of the order-disorder transition on the internal stress. The coincidence of isometric and isotonic data shows that in this case the external and internal stresses are equivalent because the isotonic curves were recorded under constant external loads. Thus, the isometric curve has an equilibrium nature, and from the onset of contraction to the critical point is a phase diagram of the system and is analogous to the pressure-temperature phase diagrams of ordinary liquids.

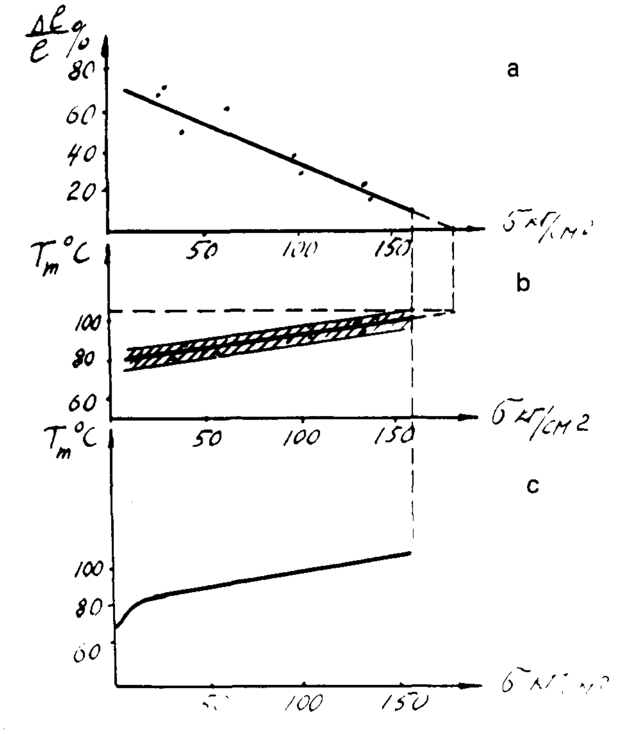

Figure 3. Thermomechanical properties of the collagen fibers in water at $\sigma < \sigma_{cr}$. (a) The dependence of the maximal contraction on stress; (b) the dependence of the transition temperature on the external and internal stress; (X) temperature-deformation data. (c) Experimental isometric curve.

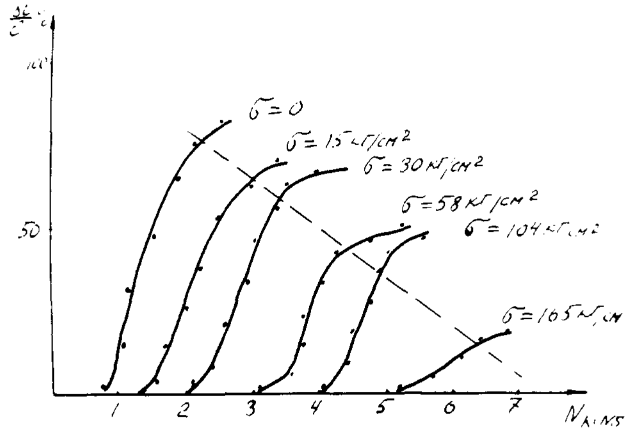

Figure 4. Contraction of collagen fibers in KCNS solutions under different fixed loads.

The similarity between phase diagrams and isometric heating curves was first mentioned by Ciferri and Smith in 1964.[9] They calculated the crystal-melt phase diagrams and the changes of elastic properties for the different crystal forms of polymers. They used the equation of Flory and Gee and reasonable estimates of the molecular parameters involved. They noted that the isometric heating

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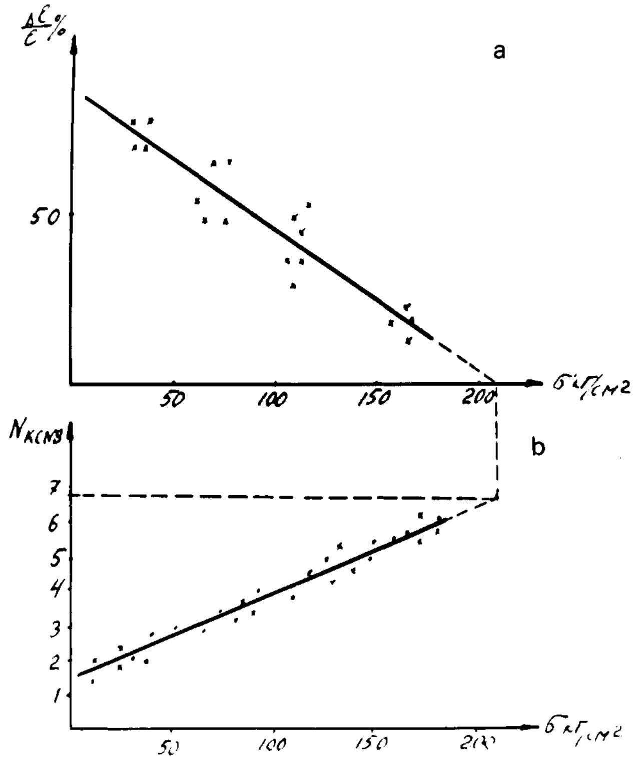

Figure 5. (a) The dependence of the maxima-1 contraction of the collagen fibers in the KCNS solution on the stress. (b) The dependence of the transition concentration on the external and internal stress: (O) isometric data; (X) isotonic data.

curves and phase diagrams coincide for some portion of the curves. Each point of a "usual" phase diagram is a point of equilibrium for two phases coexisting at a given temperature, whereas the points of isometric curves represent only the crystalline phase below the critical point and only the amorphous phase above it (if the crystalline fiber is heated). Our experimental data confirm completely the theoretical conclusion of Ciferri and Smith for the system studied.

The results of our study of the KCNS-induced contraction of collagen are entirely similar to the data described above concerning its hydrothermal shrinkage. A similar set of isotonic curves (Figure 4) was obtained and the critical stress and critical concentration of KCNS was evaluated in the same way (Figure 5a,b). The estimates of critical stress and concentration are 216 ± 15 kg/cm^2 and 6.75 mol of KCNS/l. The isometric and isotonic data also coincide completely for the KCNS contraction of collagen and everything said about the thermodynamic significance of the isometric curve is also true for this case.

The X-ray observations showed an almost total reversibility of the structural changes occuring in these transitions. Using wide- and low-angle X-ray diffraction a very close similarity was observed between the X-ray patterns of initially native collagen, of collagen contracted and then stretched to the original length, and collagen prevented from contracting by being fastened to a rigid frame. The same observations were made for chemical contraction. Hence it was found that the order–disorder transitions which occur are reversible from the point of view of the overall structure and partially so for the fine morphology of collagen.

The equilibrium nature of the isometric curve allows us to make some thermodynamic calculations. Using the above-mentioned formula of Flory, we calculated the enthalpy of hydrothermal contraction as 212 kcal/mol of peptide units. This value is in satisfactory agreement with other data.[10] Using the Katchalsky[11] equation for the chemical contraction of fibrous proteins, we obtained the value $\Delta\epsilon$-"the enrichment of salt" by polymer which is thermodynamically equivalent to the enthalpy. The estimate of this value was 4.6 × 10^{-2} mol of KCNS/mol of peptide units; this is in good agreement with the value obtained by Oplatka and coworkers[12] for this system. We also calculated the cooperativity parameters for both systems from the isotonic curves using Zimm's formula[13] for the hydrothermal contraction, and the formula[14] of Birstein and Ptitzin for the chemical one. The estimates were about 10^{-4} for both systems; this corresponds approximately to the cooperativity parameters of the Zimm–Bragg[15] or Gibbs–Di Marzio[16] theories of helix–coil transitions, 10^{-4}–10^{-5}.

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